directly assessed on isolated 2 months-old CM stimulated or not with neuregulin-1 growth factor. Remarkably, while this treatment resulted in 0.17% of BrdU uptake in WT CM, it increased up to 9.57 % in KO. KO CM also exhibited significant higher mitotic (pH3+) and cytokinesis (AuroraB+) events compared to WT. Thus, the capacity for adult KO CM to proliferate is not restricted to aged CM but more likely an intrinsic potential of young KO CM. We next assessed the proliferative capacity of 2 month-old KO mice in vivo using the apectomy model. Remarkably, while WT mice developed a classical healing process (fibrosis/inflammation), KO mice almost completely regenerated the apex as indicated by the presence of mitotic (BrdU+, pH3+, AuroraB+) CM and significant reduced fibrosis (50%) in the resected zone.

These results demonstrated that ephrin-B1 protein is a specific blocker of adult CM proliferation and its downregulation represents a huge interest for future therapeutic approaches in cardiac regenerative medicine.

0128

Sca-1 positive cells, but not c-kit positive cells, differentiate into mature cardiomyocytes after brain natriuretic peptide treatment

Stéphanie Rignault (1), Christelle Bielmann (1), Lucas Liaudet (2), Bernard Waeber (1), François Feihl (1), Nathalie Rosenblatt (1)
(1) CHU Vaudois, Physiopathologie, Lausanne, Suisse – (2) CHU Vaudois, Soins Intensifs Adultes, Lausanne, Suisse

The Brain Natriuretic Peptide (BNP) is a cardiac hormone, which promotes the recovery of cardiac function and the preservation of cardiac tissue in animal models of heart diseases. Its cardiac protective role in animals was attributed to fibrosis inhibition, as well as to reduction of cardiomyocyte apoptosis and hypertrophy. Recently, we demonstrated that BNP induces heart regeneration via the stimulation of cardiac precursor cell (CPC) proliferation and differentiation into mature cardiomyocytes.

The aim of our study was to identify which CPC's subset is able to respond to BNP stimulation.

Cardiac precursor cells identified as being Sca-1$^+$ Nkx2.5$^+$ or c-kit$^+$ Nkx2.5$^+$ cells, expressed in neonatal and adult hearts BNP's receptors (NPR-A and NPR-B), showing their ability to be activated by BNP treatment. Cell sorting experiments based on the expression of Sca-1 or c-kit were performed on non-myocyte cells isolated from neonatal wild-type hearts. Sca-1$^+$ and c-kit$^+$ cells were cultured up to 3 weeks with or without BNP in differentiating medium. Sca-1 positive cells, which contained few c-kit$^+$ cells, responded clearly to BNP stimulation by upregulating mRNA levels of genes coding for Nkx2.5, Mlc-2v, c-kit, Sca-1, beta and alpha MHC. Furthermore, higher number of Troponin I$^+$ cells was detected in BNP treated cells compared to untreated cells, suggesting that Sca-1$^+$ cells differentiated after BNP stimulation into mature cardiomyocytes. BNP treatment of c-kit$^+$ cells didn't induce the upregulation of mRNA coding for cardiomyocyte specific genes. However, we determined that c-kit positive cells spontaneously differentiated into mature cardiomyocytes during the 3 weeks of cell culture without BNP stimulation.

To determine which receptor is involved, Sca-1$^+$ cells, isolated from neonatal hearts of NPR-A or NPR-B deficient mice, were treated with BNP. The effects of BNP on wild type and NPR-A KO cells did not differ substantially. However, Sca-1$^+$ cells isolated from NPR-B deficient hearts couldn't respond anymore to BNP stimulation.

Thus, BNP specifically stimulates via NPR-B Sca-1$^+$ cell differentiation into cardiomyocytes. c-kit$^+$ cells display clearly a cardiogenic potential which is BNP independent.

0129

Adult human mononuclear clones isolated from peripheral blood can differentiate into immature cardiomyocytes

Suzanne Badoux (1), Stéphanie Rignault (1), Christelle Bielmann (1), Fabien Cusin (1), Lucas Liaudet (2), Bernard Waeber (1), François Feihl (1), Nathalie Rosenblatt (1)
(1) CHU Vaudois, Physiopathologie, Lausanne, Suisse – (2) CHU Vaudois, Soins Intensifs Adultes, Lausanne, Suisse

Breakthroughs in stem cell biology and demonstrations of the heart's endogenous regenerative capacities have incited an intense race towards cardiac regeneration, i.e. the replacement of lost myocardium after myocardial infarction. In preclinical trials, cell transplantation therapies, using adult human multipotent stem cells (e. g, hematopoietic stem cells) into infarcted myocardium has shown enhanced cardiogenesis in animal models. Nevertheless, results in clinical trials remain unsatisfactory and determining a suitable cell population that is easily harvested and improves cardiac repair is challenging.

In previous research, our lab isolated human peripheral blood mononuclear clones (PBMCs) bearing cardiac mesodermal markers, e.g. c-kit, Islet-1 or Flk-1. Potentially, these cells can differentiate within the cardiac lineage to mature cardiomyocytes and participate in heart repair.

We sought to establish an *in vitro* cardioinstructive differentiation protocol to derive cardiomyocytes from our PBMC population. For this, we screened three differentiation protocols, i.e. Keller, Smith and He protocols. To follow cell differentiation RT-qPCR and immunostainings were performed for relevant genes, e.g. Nkx2.5, GATA4, Troponin T (cTNT), β-Myosin heavy chain (β-MHC) and α-actinin. Results for mRNA expression showed that at least 75% of PBMCs can derive to a cardiac precursor population (Nkx2.5$^+$/GATA4$^+$) following Keller and Smith protocol. In Smith protocol, 75% of PBMCs differentiated to immature cardiomyocytes (cTNT$^+$) of which 50% expressed both cTNT and β-MHC when co-cultured with neonatal mice ventricular myocytes. Immunofluoresence assay showed that PBMCs in both Keller and Smith protocol are Nkx2.5$^+$/α-actinin$^+$ demonstrating differentiation at the protein level.

Next we evaluated the safety, survival and integration of injected PBMCs in neonatal Gt(ROSA)^{26}-Tomato mice tissues *in vivo*. By staining PBMC with membrane marker PKH2GL, we followed cell homing within the animal tissues visualized by fluorescent microscopy on tissue sections.

In conclusion, we demonstrated that human PBMCs can differentiate, under certain conditions, into cardiac precursor cells or immature cardiomyocytes *in vitro*. These results make them an interesting and promising cell source for stem cell therapies in cardiac repair.

0090

Human cardiac progenitor cell seeded-collagen patches for cell therapy applied to right ventricular dysfunction: preliminary results in a large animal model

Catherine Rucker-Martin (1), Amir Hodzic (1), David Luu (2), Mathieu Coblence (3), Mohamed Ly (2), Emmanuel Le Bret (2), Elodie Gouadon (1), Marc Humbert (1), Philippe Jourdon (4), Michel Pucéat (4), Virginie Lambert (2)
(1) Centre Chirurgical Marie Lannelongue, INSERM U999, Le Plessis Robinson, France – (2) Centre Chirurgical Marie-Lannelongue, Cardiopathies Congénitales, Le Plessis Robinson, France – (3) Centre Chirurgical Marie Lannelongue, Laboratoire de Recherche Chiruirgical, Le Plessis Robinson, France – (4) Faculté de Médecine, INSERM UMR-S910, Team physiopathology of cardiac development, Marseille, France

Background: Cell therapy using intramyocardial injections of cardiac progenitors issued from human embryonic stem cells showed benefits on overloaded right ventricular (RV) tissue remodelling and arrhythmic susceptibility but this delivery mode failed to improve RV function. Our aim was to evaluate in a porcine model of overloaded RV dysfunction a new delivery mode of such therapy.

Methods: A combined overloaded RV dysfunction was obtained in piglets using a surgical procedure mimicking repaired tetralogy of Fallot. After 4 months, cell therapy was surgically administrated using 2 types of human NKX2.5+ cardiac progenitor cell-seeded collagen patches applied on the epicardium: QGel® and pressured-patches. Myocardial function was measured 1 month after transplantation by conductance catheter technique and echocardiography (standard and strain). The fate of progenitors was studied using antibodies directed against Ki67, CD31, actinin and Islet1.

Results: All pigs survived without any complication. Pressured-patches allowed human progenitors to migrate across the complete myocardium while QGel® patches restricted the cell migration to only a third of the myocardium. In both cases, progenitors differentiated toward the cardiac lineage assessed by Islet1 and actinin expression and maintained their proliferation capacity. Concerning RV function, only pressured-patches (N=3) tended to improve the contractility (Emax slope). By contrast, this parameter decreased in QGel® patches animals (N=2). Moreover, in 2 pressured-patch animals, standard echocardiographic functional parameters (FAC, TAPSE, s'wave) were maintained while 2D strain and strain rate values increased.

Conclusion: Cell therapy using seeded-patches was more conservative for engrafted cells than intramyocardial injections but only pressured-patches seemed to give benefits on overloaded RV function and contractility. These first promising results need to be checked at longer term.